

## REMARKS

Claims 1 - 25 are pending in the present application.

Applicants have now submitted a Sequence Listing and a corresponding computer-readable Sequence Listing. Sequence Identifiers (SEQ ID NO:) have been added to the specification. The sequence information recorded in the corresponding computer-readable Sequence Listing is identical to the paper copy of the Sequence Listing. Support for all of the sequences listed in the Sequence Listing is found in the present application as originally filed. No new matter is believed to have been introduced by the submission of the Sequence Listing and the corresponding computer-readable Sequence Listing.

Applicants submit that the present application is ready for examination on the merits. Early notice to this effect is earnestly solicited.

Respectfully submitted,

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Docket No.: 200805US55

Serial No: 09/732,754

Amendment Filed: 09/25/01

**IN THE SPECIFICATION**

Please amend the specification as follows:

Please replace the paragraph beginning on page 9, line 25, with the following text:

--The antigenic peptide used in this experiment had the following sequence:

H-K(PAM)TT-pol 476-484

Nh2-K(NePam)GRQYIKANSKFIGITERGRILKEPVHGV-COOH (SEQ ID NO:1).--

Please replace the paragraph beginning on page 12, line 6, with the following text:

--Synthetic lipid- and non-lipid-tailed polypeptides

The amino-Acid sequences were LSA3-NRII Ac-

LEESQVNDDIFNSLVKSVQQEQQHNVK(Pam)NH2 (SEQ ID NO:2) and LSA1-J Ac-

ERRAKEKLQEQQSDLEQRKADTKKK(Pam)NH2 (SEQ ID NO:3). in which the lipid-tail was covalently linked to the side chain of a C-terminal lysylamide residue. These lipid-tailed polypeptides were as previously described (Fidock et coll., 1994; BenMohamed et coll. 1997; Perlaza et coll., 1998). Most polypeptides and lipopeptides were >90% pure, as determined by HPLC.--

Please replace the paragraph beginning on page 12, line 28, with the following text:

--Individual blood samples were obtained *via* the retro-orbital plexus by 9 to 15 days post immunization (dpi) and sera were stored at [- 70;C] -70°C until assayed for IgG, IgA and IgM polypeptide- and parasite- specific Abs. The presence of anti-peptide antibodies in sera was determined using Enzyme-linked immunosorbent assay (ELISA) as reported

previously (BenMohamed et coll., 1997). ELISA plates (Nunc, Roskilde, Denmark) were coated overnight at [4;C] 4°C with 0.1 ml of LSA3-NRII (SEQ ID NO:2) or LSA1-J (SEQ ID NO:3) polypeptide solution at 3 µg/ml in PBS buffer pH 7.4 containing 3% BSA. The LSA1-J (SEQ ID NO:3) polypeptide was used as the irrelevant control of LSA3-NRII (SEQ ID NO:2) and *vice versa*. The plates were washed twice in PBS with 0.01% Tween-20 (PBS-T), blocked for 1 hr in PBS-T supplemented with 1% BSA prior to the addition of 0.1 ml of 1/100 dilution of mouse sera. The plates were then incubated at [37;C] 37°C for one hour. After washing, the bound IgG were detected using peroxidase-conjugated goat anti-mouse IgG (Biosys, Compiègne, France) added at a 1/2000 dilution. Following incubation at [37;C] 37°C for 1 hour and a final wash, 50 µl of 0.30 % H<sub>2</sub>O<sub>2</sub> containing orthophenylenediamine dihydrochloride (OPD, Sigma, St. Louis), dissolved in 0.1 M citrate buffer (pH 5.0) were added to each well at room temperature. The OD450 nm was measured using a multichannel spectrophotometer (Titertek Multiskan MCC. 340). Individual sera from all groups were diluted 1/100 and analyzed separately. Preimmune sera were used as negative controls and the results were expressed either as optical density (OD) at 450nm or as ELISA-RATIO calculated as follows: OD450 nm postimmune sera divided by OD450 nm preimmune sera. For polypeptide-specific ELISAs, sample dilution were considered positive if the OD450 nm recorded for that dilution was at least twofold higher than the OD450 nm recorded for a naive sample at the same dilution (Fidock et coll., 1994 ; Bottius et coll., 1996). Isotype analysis of mouse was carried out using class specific alkaline phosphatase-conjugated Goat anti-Mouse IgA, IgM, IgG1, IgG2a, IgG2b or IgG3 HRP-Labeled (Southern Biotechnology Associates, Birmingham, USA) added at a 1/2000 dilution in PBS-T, as previously described (BenMohamed et coll., 1997).--

Please replace the paragraph beginning on page 14, line 2, with the following text:

--For proliferation assays, spleen and inguinal lymph nodes were obtained from mice (3 to 6 per group) on 14 dpi using sterile forceps and placed into ice-cold Hank's balanced salt solution (HBSS). Single-cell suspensions were prepared by crushing the tissues between the frosted ends of two microscope slides. Red blood cells were removed by treatment with ammonium chloride on ice for 10 min. The single-cell suspensions were washed twice in RPMI-1640 (Gibco, Courbevoie, France) and were adjusted to  $4 \times 10^6$  cells/ml in RPMI-1640 media supplemented with 1.5% heat-inactivated fetal calf serum (FCS), 1% penicillin-streptomycin, 1% glutamine,  $5 \cdot 10^{-5}$  M 2- $\beta$ -mercaptoethanol (Gibco), and 1% *N*-n-hydroxyethylpiperazine-*N'*-2 ethanesulphonic acid (HEPES), pH 7.4, and used as previously described (BenMohamed et coll., 1997). Equal volumes of cells and complete medium or complete medium with LSA3-NRII (SEQ ID NO:2) or LSA1-J (SEQ ID NO:3) polypeptides were mixed to give a final concentration of  $2 \times 10^6$  cells/ml in medium alone or in medium with polypeptide at 90, 30, 10, 3, or 1 mg/ml. The cell suspensions were incubated for 72h at 37°C and 7.5% CO<sub>2</sub>. Three days later, one  $\mu$ Ci of tritiated deoxythymidine ((3H)TdR) (Amersham, Les Ulis, France) was added to each well, for 16h before the cultures were harvested (Skatron, Lierbyen, Norway) and the incorporated radioactivity determined by liquid scintillation (LKB-Wallac, Turku, Finland). Results are expressed as the mean cpm of cell-associated (3H)TdR recovered from wells containing Ag, subtracted by the mean cpm of cell-associated (3H)TdR recovered from wells without Ag (D cpm) (average of triplicates). The results were considered positive when the D cpm is  $\geq$  to 1000 cpm and stimulation index  $> 2$  (Fidock et coll., 1994 ; Bottius et coll., 1996 ; BenMohamed et coll., 1997).--

### **IN THE CLAIMS**

Please amend the Claims as follows:

--14. (Amended) The lipopeptide of Claim 12, wherein the lipopeptide is [: LSA3-NRII Ac-LEESQVNDDIFNSLVKSVQQEQQHNVK(PAM)NH2 OR LSA1-J Ac-ERRAKEKLQEQQSDLEQRKADTKKK(PAM)] (SEQ ID NO:2) or (SEQ ID NO:3).

15. (Amended) The method of Claim 9, wherein the lipopeptide is [: LSA3-NRII Ac-LEESQVNDDIFNSLVKSVQQEQQHNVK(PAM)NH2 OR LSA1-J Ac-ERRAKEKLQEQQSDLEQRKADTKKK(PAM)NH2] (SEQ ID NO:2) or (SEQ ID NO:3).--

--22. (Amended) The method of Claim 21, wherein the lipopeptide is an antigenic lipopeptide of [sequence:

H-K(PAM)TT-pol 476-484

Nh2-K(NePam)GRQYIKKANSKFIGITERGRILKEP-COOH] (SEQ ID NO:1).--



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